

Calypte HIV-1 BED Incidence EIA Troubleshooting Guide

Observed Problem:

- OD of a Single Control or Calibrator out of range [Page 1](#)
- All controls and the Calibrator have ODs that are too high or too low [Pages 2, 3, 4, 5](#)
- There is no OD signal for any control, calibrator, or sample [Pages 5, 6](#)
- Crystals appear in wash buffer concentrate [Page 6](#)
- High occurrence of samples that were classified as “recent infections” by screen test but “long term infections” after confirmation test [Page 6](#)
- Test strips remain but some reagents have become exhausted [Page 7](#)

Problem	Possible cause	Solution
OD of a Single Control or Calibrator out of range.	Wrong control/calibrator used.	Pay special attention to color coded vial labels to ensure that the correct control or calibrator is used.
	Improper dilution of control/calibrator.	Controls are diluted 1:101 by the addition of 5µL control to 500µL sample diluents.
	Inadequate mixing of diluted control/calibrator.	Ensure that controls are properly mixed as specified in the product insert.
	Contamination has occurred due to vial caps having been switched.	Do not switch caps on controls and/or calibrator.
	Plate washer has a clogged pin leading to inadequate washing of a single well.	Ensure that all pins on the manifold are clear of debris so that each well may be washed.
	Micropipettes not calibrated.	A pipetting error of only 1µL due to micropipettes out of calibration can lead a control value that is out of specification.
All controls and the Calibrator have ODs that are too high or too low.	One or more incubation time was not adhered to.	Incubation times must be strictly adhered to.
	Temperature of 37°C and/or 25°C incubator too high or too low.	Temperature of incubations must be strictly adhered to (37°C for all steps except substrate at 25°C). Ensure that the thermometer display on the incubator is accurate by using a calibrated thermometer.
	Sample diluent prepared incorrectly.	Ensure that the sample diluent was prepared by adding 3 grams BSA for every 100 mL of 1X wash buffer.
	1X Wash buffer prepared incorrectly.	Ensure that the 1X wash buffer was prepared by diluting the 10X wash buffer stock by 1:10 (e.g. adding 100 mL to every 900 mL water).
	Buffer other than the wash buffer was used to wash the plate.	Many plate washers have various channels controlled by valves. Make sure that the Incidence Test wash buffer is being used to wash the plate.
	Plate washer was not adequately primed prior to use.	Ensure that the plate washer is primed to remove any residual wash buffer, cleaning solution, or bleach that may have been in the hoses.

Problem	Possible cause	Solution
All controls and the Calibrator have ODs that are too high or too low (<i>continued</i>).	Plate washer malfunction.	Check to make sure the plate washer is programmed correctly and is adequately washing the plate. A clogged manifold, and incorrect plate height, aspiration and dispensing adjustments will also lead to incomplete washing.
	Bleach in plate washer waste container interfering with assay/wash buffer.	Remove bleach from waste container. Remember to disinfect the waste prior to disposal.
	Plate reader out of calibration.	Ensure that plate reader has been recently calibrated and checked against a reference plate.
	HIV-BED peptide and/or conjugate solution prepared with an incorrect dilution or in wrong buffer, and/or with inappropriate pipetting instrument.	Ensure that the HIV BED and conjugate concentrates are diluted 1:1001 (1µL per 1mL 1X sample diluent) using appropriate micropipette (p10 or p20).
	Incorrect amount of sample or test reagent added to the wells.	Each procedural step (sample, peptide, conjugate, substrate, and stop solution) requires the transfer of 100µl of reagent.
	Freezer pack and refrigerator packs from separate lots mismatched during testing.	The freezer pack contains components that are specifically matched with the particular refrigerator pack constituting a kit lot. Ensure that only the components from one kit lot are used during testing.
	Cross-contamination has occurred due to spillage, failure to change pipette tips, contaminated reagent reservoirs, etc.	Follow proper laboratory technique and use clean supplies while performing the test to prevent cross-contamination.
	Improper dilution of controls/calibrator.	Controls are diluted 1:101 by the addition of 5µL control to 500µL sample diluents.
	In adequate mixing of diluted controls/calibrator.	Ensure that controls are properly mixed as specified in the product insert.
	Micropipettes not calibrated.	Use only calibrated micropipettes. A 1µL pipetting error of controls, BED peptide, or conjugate due to micropipettes out of calibration can lead to higher or lower ODs.

Problem	Possible cause	Solution
All controls and the Calibrator have ODs that are too high or too low (<i>continued</i>).	TMB substrate contaminated.	If the TMB is contaminated it will have a blue color and will elevate all OD values. Discard any contaminated TMB.
	Temperature of TMB (room temperature) is significantly less than 25°C.	If room temperature is significantly less than 25°C the TMB will be too cool during the first several minutes of substrate incubation and thus the reaction and signal will be suppressed. If the room temperature is cool, maintain the TMB in the 25°C incubator until use.
	If kit components were not at room temperature when used, OD values could be lower.	Ensure that all kit components are at room temperature prior to use.
	Plate not stopped with stop solution (1N H ₂ SO ₄).	Substrate reaction must be stopped with the addition of 100µl of stop solution.
	Plate read with incorrect filters.	Plate should be read at 450nm with reference of 630nm to 650nm.
	Plate Reader filter associations incorrect.	Check the plate reader with a test plate to ensure that filter associations have not been changed.
	Test kit or components were stored inappropriately at laboratory.	Freezer pack components must be held at or below -20°C. Refrigerator pack components must be held at 2-8°C.
	Expired kit used for testing.	Ensure that kit is used within expiration date.
	Plate bag not properly sealed after last use.	Ensure that unused strips are stored in the foil pouch, that the desiccant is still present, and that the pouch is zip sealed.
	Unclean water used to prepare reagents.	Use only clean water to prepare wash buffer and sample diluent. Chemical, organic, and/or microbial contaminants can interfere with the assay.
Inadequate mixing of vial components prior to use.	Vortex each vial component prior to use as described in the product insert.	
Inadequate mixing of diluted controls/calibrator.	Ensure that controls are properly mixed as specified in the product insert.	

Problem	Possible cause	Solution
All controls and the Calibrator have ODs that are too high or too low (<i>continued</i>).	Previously prepared specimen diluent used after 2 days following its preparation.	Discard any unused specimen diluent after 2 days.
	Reagent reservoirs are contaminated due to reuse.	Discard reagent reservoirs after single use to prevent contamination.
	Plate has been exposed to bleach fumes from countertop.	Allow bleach fumes to dissipate from laboratory test area prior to running the assay.
There is no OD signal for any control, calibrator, or sample	Too much time passed between when the plate was stopped and when the plate was read on the plate reader.	After stopping the plate with stop solution, the plate must be read immediately.
	Specimens and controls not added to sample diluent.	Ensure that specimens and controls are added to the titer tubes containing sample diluents.
	A procedural step such as addition of BED peptide or conjugate was skipped or executed in the incorrect sequence.	All procedural steps must be executed and followed in the correct sequence for the assay to work.
	Some liquid other than TMB used as the substrate.	Ensure that the substrate (TMB) is used at the appropriate step.
	Buffer other than the wash buffer was used to wash the plate.	Many plate washers have various channels controlled by valves. Make sure that the Incidence Test wash buffer is being used to wash the plate.
	HIV-BED peptide and/or conjugate solution prepared with an incorrect dilution or in wrong buffer.	Ensure that the HIV BED and conjugate concentrates are diluted 1:1001 (1µL per 1mL 1X sample diluent).
	Plate washer was not adequately primed prior to use.	Ensure that the plate washer is primed to remove any residual wash buffer or cleaning solution that may have been in the hoses.
	Bleach in plate washer waste container interfering with assay/wash buffer.	Remove bleach from waste container. Remember to disinfect the waste prior to disposal.
	Plate not stopped with stop solution (1N H ₂ SO ₄).	Substrate reaction must be stopped with the addition of 100µl of stop solution.

Problem	Possible cause	Solution
There is no OD signal for any control, calibrator, or sample (<i>continued</i>).	Plate read with incorrect filters.	Plate should be read at 450nm with reference of 630nm to 650nm.
	Plate Reader filter associations incorrect.	Check the plate reader with a test plate to Ensure that filter associations have not been changed.
	Plate reader out of calibration.	Ensure that plate reader has been recently calibrated and checked against a reference plate.
	Test kit or components were stored inappropriately at laboratory.	Freezer pack components must be held at or below -20°C. Refrigerator pack components must be held at 2-8°C.
	Expired kit used for testing.	Ensure that kit is used within expiration date.
	Improper dilution of controls/calibrator.	Controls are diluted 1:101 by the addition of 5µL control to 500µL sample diluents.
	Inadequate mixing of vial components prior to use.	Vortex each vial components prior to use as described in the product insert.
	Unclean water used to prepare buffers.	Use only clean deionized water to prepare wash buffer and sample diluent. Chemical, organic, and/or microbial contaminants can interfere with the assay.
	Plate has been exposed to bleach fumes from countertop.	Allow bleach fumes to dissipate from laboratory test area prior to running the assay.
Crystals appear in wash buffer concentrate.	Wash buffer concentrate has not been warmed to room temperature.	Only prepare wash buffer with wash buffer concentrate that is at room temperature.
High occurrence of samples that were classified as “recent” by screen test but “long term” after confirmation test.	If a sample was not added to the diluent during the initial test (or added incorrectly), it will erroneously appear as a “recent” infection. Inadequate mixing will also lead to erroneous screening results.	Ensure that every sample is added to the appropriate titer tube and that each tube is adequately mixed.

Problem	Possible cause	Solution
Test strips remain but some reagents have become exhausted.	Buffers or working solutions are being prepared incorrectly.	If buffers and solutions are prepared correctly, there should be an adequate amount of reagents for every strip. Ensure that buffers are being prepared correctly. Reagents from one kit may be used with another kit only if they share the same lot number.
	Excessive amounts of buffers or working solutions are being prepared.	Prepare buffers and solutions in only the amount needed for the current test. Reagents from one kit may be used with another kit only if they share the same lot number.